1083-8791/07/1308-0001$32.00/0
doi:10.1016/j.bbmt.2007.04.001

ASBMT American Society for Blood and Marrow Transplantation

Posttransplant Thrombopoiesis Predicts Survival in Patients Undergoing Autologous Hematopoietic Progenitor Cell Transplantation

Mary J. Ninan,[1,2] Christopher R. Flowers,[2] John D. Roback,[2] Martha L. Arellano,[2] Edmund K. Waller[2]

[1]Atlanta Medical Center, Atlanta, Georgia; and [2]Bone Marrow and Stem Cell Transplant Center, the Winship Cancer Institute, Emory University, Atlanta, Georgia

Correspondence and reprint requests: Edmund K. Waller, MD, PhD, Department of Hematology and Oncology, Winship Cancer Institute, Emory University, 1365C Clifton Road, Room C4002, Atlanta, GA 30322 (e-mail: ewaller@emory.edu).

Received January 19, 2007; accepted April 6, 2007

ABSTRACT

The frequency and clinical significance of secondary thrombocytopenia following initial engraftment in autologous hematopoietic progenitor cell transplantation (HPCT) is unknown. An institutional review board approved retrospective study of thrombopoiesis was performed in 359 patients transplanted with autologous blood (97%) or marrow (3%) who achieved platelet engraftment to >50,000/μL. Idiopathic secondary post-transplant thrombocytopenia (ISPT) was defined as >50% decline in blood platelets to <100,000/μL in the absence of relapse or sepsis. ISPT occurred at a median of day +35 posttransplant in 17% of patients. Patients with ISPT had similar initial platelet engraftment (median 17 days) versus non-ISPT patients (18 days; *P* = NS) and recovered platelet counts (median 123,00 K/μL) by day 110 posttransplant. Four factors were independently associated with post-transplant death in a multivariate model: disease status at transplant; the number of prior chemotherapy regimens, failure to achieve a platelet count of >150,000/μL posttransplant, and the occurrence of ISPT. A prognostic score was developed based upon the occurrence of ISPT and posttransplant platelet counts of <150,000/μL. Survival of patients with both factors (n = 25) was poor (15% alive at 5 years); patients with 1 factor (n = 145) had 49% 5-year survival; patients with 0 factors (n = 189) had 72% 5-year survival. Patients who failed to achieve a platelet count of >150,000/μL received significantly fewer $CD34^+$ cells/kg (*P* < .001), whereas patients with ISPT received fewer $CD34^+CD38^-$ cells/kg (*P* = .0006). The kinetics of posttransplant thrombopoiesis is an independent prognostic factor for long-term survival following autologous HPC. ISPT and lower initial posttransplant platelet counts reflect poor engraftment with long-term and short-term repopulating $CD34^+$ hematopoietic stem cells, respectively, and are associated with an increased risk of death from disease relapse.

KEY WORDS

Thrombopoiesis • Stem cells • Platelets • Thrombocytopenia • Autologous transplantation

INTRODUCTION

Profound thrombocytopenia occurs in all patients following myeloablative conditioning and autologous stem cell rescue, and reflects bone marrow aplasia induced by high-dose chemotherapy and/or total body irradiation (TBI). Following autologous hematopoietic progenitor cell transplantation (HPCT), restoration of normal numbers of blood leukocytes and platelets usually follows predictable kinetics within the first month posttransplant, with increasing numbers of granulocytes, platelets, lymphocytes, and finally erythrocytes in the blood. The kinetics of granulocyte recovery have been extensively studied in autologous transplant recipients leading to identification of the minimum and optimal numbers of $CD34^+$ hematopoietic progenitors necessary for predictable reestablishment of hematopoiesis [1-3]. Higher numbers of CD34 cells in the graft are associated with more rapid neutrophil and platelet engrafment [4-6] but not necessarily better long-term outcomes [7]. The recovery of a higher number of blood lymphocytes early post-transplant has been reported to be associated with

improved posttransplant survival, and appears to be correlated with transplantation of larger numbers of lymphocytes in the hematopoietic graft rather than de novo lymphopoiesis from lymphocyte progenitors in the graft [8-12].

In contrast, studies of the kinetics of platelet recovery following HPCT have largely been limited to descriptions of the time from transplant to the day of independence from platelet transfusions. Most reports describing clinical outcomes following autologous transplantion have compared the median time the blood platelet counts reached 20,000/μL or 50,000/μL without tranfusion support [13]. Although the availability of myeloid and erythroid growth factors offer safe and effective management strategies for mitigating posttransplant neutropenia and anemia, respectively, similar agents are not currently commercially available for the management of posttransplant thrombocytopenia [14]. Thus, the platelet count following autologous HPCT represents a direct indicator of the hematopoietic activity of the graft.

A subset of patients who initially recover platelet counts after transplantation experience subsequent secondary posttransplant thrombocytopenia (SPT), or varying duration, and because of different mechanisms [15]. Sepsis or relapse may result in early SPT; SPT occurring 6-12 months posttransplantation has been managed with steroids and immune globulin, drugs that are used to treat patients with idiopathic thrombocytopenia purpura [16,17]. The phenomenon of idiopathic secondary posttransplant thrombocytopenia (ISPT), ocurring within the first 100 days posttransplant, has not been well defined.

To define the incidence of ISPT, its association with pre- and posttransplant variables, the kinetics of platelet production following autologous transplantation, and the prognostic significance of posttransplant thrombopoiesis, we undertook a retrospective study of platelet production in consecutive patients undergoing autologous HPCT at a single institution. We hypothesized that an idiopathic secondary decline in blood platelet counts following initial engraftment would be associated with transplantation of a hematopoietic graft containing fewer numbers of phenotypically undifferentiated $CD34^+$ $CD38^-$ hematopoietic progenitors [18,19] responsible for long-term repopulating activity [20].

METHODS

Study Design and Definitions

We performed a single institution retrospective cohort study on platelet engraftment kinetics and survival among consecutive patients undergoing high-dose chemotherapy and autologous hematopoietic progenitor cell transplantation. The day of granulocyte engraftment was defined as the first of 3 consecutive days on which patients had an absolute granulocyte count of at least 500/μL. The day of platelet engraftment was defined as the day platelet counts ≥50,000/μL were achieved without a platelet transfusion in the previous 7 days. Because there are no existing descriptions of the phenomena of SPT (outside of late posttransplant thrombocytopenia), SPT was defined as a clinically significant drop in platelet count, of at least a 50% absolute decrease, to a value <100,000/μL after initial platelet engraftment achieved. ISPT was defined as SPT within the first 100 days posttransplant in the absence of relapse or sepsis. Demographics of the patients, their diagnoses, conditioning regimens, and characteristics of their grafts were entered into a study-specific, institutional review board (IRB)-approved database along with blood counts, transfusions, and infections occurring within the first 100 days posttransplant. Follow-up survival data was available for surviving study subjects for a median of 2 years posttransplant.

Study Subjects

The study set was derived from 509 consecutive cancer patients who underwent high-dose chemotherapy and autologous blood (97%) or bone marrow (3%) HPC transplantation between January 1997 and June 2005. An IRB-approved waiver of consent was granted for the conduct of this study. Forty patients had insufficient data on posttransplant platelet counts to assess engraftment, 6 patients died prior to engraftment (1% early death), and 18 patients did not achieve a platelet count of >50,000/μL by day +100 (4% failure to achieve platelet engraftment). Three hundred fifty-nine of the 445 remaining patients (81%) had data on the $CD34^+$ cell subsets in the autograft and constituted the study cohort. Survival was documented at posttransplant follow-up visits, or by contacting the patient or referring oncologist. The date of death was confirmed using Social Security Death Index data. The primary cause of death was available for 78% of deceased patients. Treatment regimens were defined as chemotherapy protocols given as part of a planned course of therapy (ie, ABVD); induction chemotherapy and mobilization with 1 cycle of cyclophosphamide given at the end of 1 course of therapy (ie, VAD) was considered to be a single regimen.

Statistical Methods

All statistical analyses were performed using SPSS version 11.0 (SPSS Inc., Chicago, IL). Comparisons between mean values occurred using a Student's *t*-test. Comparisons of ordinal characteristics between ISPT and controls were performed using a chi-square test. Survival differences between subgroups were compared using the Kaplan-Meier estimate of survival and

log rank comparisons, with univariate and multivariate Cox regression models.

RESULTS

Posttransplant Platelet Counts Were Biphasic

Inclusion of patients in this retrospective study was limited to patients who engrafted with a platelet count of at least 50,000/μL posttransplant; all 359 patients who engrafted with platelets also engrafted with granulocytes. Among the 359 study subjects, 73 (20%) met the criteria for SPT within the first 100 days posttransplant, achieving a transfusion-independent platelet count of ≥50,000/μL followed by a ≥50% decline in their platelet count to a value of <100,000/μL. Of these, 11 cases of SPT resulted from relapse within the first 100 days (6 cases) or sepsis (5 cases), leaving 62 patients (17%) with ISPT, and 297 control patients who did not experience ISPT.

Median values for serial blood platelet counts in the 62 patients with ISPT were compared to 62 patients selected from the 297 patients of who did not meet the criteria for ISPT (controls) and are shown in Figure 1A. The subset of controls and the cases had similar ages (mean ± SE) of 44 ± 1.8 years and 48 ± 1.8 years, respectively, P = NS, and a similar distribution of diagnoses (eg, 58% lymphoma patients versus 61% lymphoma patients, P = NS). Pretransplant platelet counts were similar comparing patients with ISPT to controls, with median values (±SE) of 184,000/μL (±12,000/μL) and 195,000/μL (±11,000/μL), respectively (P = NS). Initial posttransplant nadir median values (±SE) for platelet counts were 8000/μL (±600/μL) at median of day 7 posttransplant in the 62 patients with ISPT versus 8000/μL (±500/μL) at median of day 8 posttransplant in the group of 297 control patients (P = NS). Platelet engraftment to 50,000/μL occurred slightly earlier among patients in the ISPT group (median ± SE of 17 ± 1.6 days) versus control patients (median 18 ± 0.7 days; P = NS). The kinetics of initial platelet recovery was similar between the 2 groups, with a rapid rise to maximal median values (±SE) of 168,000/μL (±9000/μL) on day 20 (±2.3 days) in ISPT patients versus 191,000/μL (±6000/μL) on day 23 (±1 days) posttransplant in the group of control patients, P = .01. The initial rise in platelet counts was followed by a secondary decline in both groups. However, the decline in patients with ISPT was much more profound, falling to a nadir of 35,000/μL (±4000/μL) on day 35 (±2) posttransplant 14 ± 1 days after the initial peak (Figures 1A and B). Most patients with ISPT experienced a subsequent partial recovery of blood platelet counts to a median value of 123,000/μL by days 100-110 posttransplant (Figure 1A).

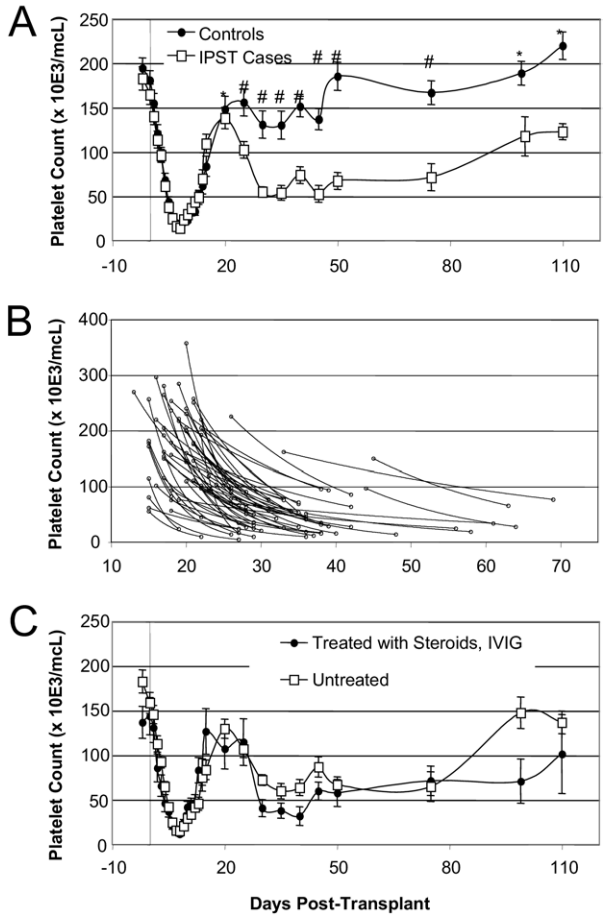

Figure 1. Comparison of platelet engraftment kinetics in patients with ISPT and controls. A, The median values (±SE) for platelet counts for 62 patients with ISPT (open squares) and 61 patients without ISPT (filled circles) are shown. After day 15, data are shown as the median of the highest platelet count recorded for each patient in a 5-day period (for days 15-50) or a 25-day period (for days 50-100). Significant differences between the groups for each time point are shown: *P < .05; #P < .001. B, Each line represents an exponential curve representing the decline from the highest recorded value for a postengraftment platelet count to the lowest posttransplant platelet count in each of the 62 patients with ISPT. C, The median values (±SE) for platelet counts for 13 patients with ISPT who received steroids + i.v. immunoglobulin (filled circles) and 49 patients with ISPT who did not receive steroids or immunoglobulin (open squares) are shown. After day 15, data are shown as the median of the highest platelet count recorded for each patient in a 5-day period (for days 15-50) or a 25-day period (for days 50-100). There were no significant differences in the mean platelet values between the 2 groups.

ISPT Was More Common in Lymphoma Patients and Patients Treated with Busulfan-Containing Regimens

The demographics of the 62 ISPT patients and the 297 control patients were similar, with a mean age of 50 years for both groups and similar sex distributions (58% and 56% male, respectively; Table 1). Hodgkin's lymphoma and non-Hodgkin's

Table 1. *Comparison of Clinical Variables between Cases with ISPT and Patients Who Did Not Develop ISPT (Controls)*

	Cases ISPT (n = 62)	Controls No ISPT (n = 297)	*P*-value
Age (mean)	53	52	NS
Male sex	36 (58%)	167 (57%)	NS
Diagnosis			.001
HD (n = 59)	18 (29%)	41 (14%)	
NHL (n = 81)	20 (32%)	61 (21%)	
MM (n = 164)	16 (26%)	148 (50%)	
Acute leukemia (n = 18)	6 (10%)	12 (4%)	
Chronic leukemia (n = 1)	1 (2%)	0 (0%)	
Solid tumor (n = 37)	1 (2%)	36 (12%)	
Number of prior regimens			NS
None	0	1 (0.3%)	
One	25 (40%)	160 (54%)	
Two	25 (40%)	106 (46%)	
Three	11 (18%)	27 (9%)	
Four	1 (2%)	3 (1%)	
Preconditioning radiation	3 (5%)	21 (7%)	NS
Preconditioning rituximab	7 (11%)	8 (3%)	.002
Disease status at transplant			NS
CR	28 (45%)	95 (32%)	
PR	29 (47%)	176 (59%)	
Refractory	5 (8%)	25 (8%)	
Untreated	0	1 (0.3%)	
Conditioning regimen			.001
Bu/Cy	5 (8%)	12 (4%)	
Bu/Cy/VP 16	40 (65%)	92 (31%)	
Melphalan	14 (23%)	144 (48%)	
Cy/TBI	2 (3%)	8 (3%)	
Cy/VP16/TBI	0	6 (2%)	
Cyt/Thio/Carbo	1 (2%)	33 (11%)	
Carbo/VP16	0	1 (2%)	
HPC source			NS
BM HPC	0	7 (2%)	
Blood HPC	61 (98%)	288 (97%)	
BM & PBSC	1 (1%)	2 (1%)	

ISPT was observed more frequently among patients with a diagnosis of lymphoma and acute leukemia, and patients who received busulfan-based conditioning. ISPT was observed less frequently in patients with solid tumors and multiple myeloma, and patients who received melphalan or STAMP V conditioning regimens.

BM indicates bone marrow; PBSC, peripheral blood stem cells; NHL, non-Hodgkin's lymphoma; MM, multiple myeloma; ISPT, idiopathic secondary posttransplant thrombocytopenia; CR, complete remission; PR, partial remission; TBI, total body irradiation; HPC, hematopoietic progenitor cell.

lymphoma were overrepresented among the group with ISPT, as were patients with prior treatment with rituximab and busulfan-based conditioning regimens (Table 1). In contrast, ISPT occurred less frequently in myeloma patients receiving high-dose melphalan conditioning and in breast cancer patients. ISPT was not significantly associated with the disease status at the time of transplant, the use of involved field radiotherapy immediately before the initiation of the conditioning regimen, or the use of bone marrow versus blood HPC grafts (Table 1).

Thrombocytopenia in Patients with ISPT Did Not Respond to Steroid or IVIG Therapy

One view of SPT is that it is immune-mediated [17]. The subset of 13 patients with ISPT that received treatment with steroids ± IVIG had similar kinetics of platelet recovery to ISPT patients who were not treated (Figure 1C). These 13 treated ISPT patients received an average of 27 days of prednisone (range: 7-43 days) at an average initial dose of 75 mg (range: 60-120 mg). The average total dose of prednisone over circa 1 month of therapy was 1295 mg (range: 400-2320 mg). Three patients received IVG at an average dose of 1500 mg/kg delivered over 3-4 days. Bone marrow examinations were performed on a minority of patients in this study as part of restaging done in some patients at day 30 posttransplant. Nineteen patients underwent bone marrow biopsies prior to day +100 in the group of non-ISPT patients; in 4 cases early relapse was detected; in an additional 15 cases (5% of the control patients) biopsies performed at a median of 35 days posttransplant revealed no cancer; these samples were evaluated for cellularity and megakaryocyte content, and were compared to bone marrow biopsies from 26 patients with ISPT (42%) who underwent biopsies at a median of 35 days posttransplant. Although the small number of samples limits the statistical power of these analyses, there was no difference in the cellularity or megakaryocyte content of the bone marrow in the group of 15 non-ISPT patients who underwent bone marrow biopsies compared to the 26 ISPT patients who underwent bone marrow biopsies (Table 2). The content of megakaryocytes in the bone marrow in patients with ISPT was not significantly increased (as often is seen in patients with ITP) compared to bone marrow biopsies in control patients (Table 2). Of note, severe hemorrhage was rare in the autotransplant population at our institution, and was listed as a cause of death in only a single patient (non-ISPT group) in this study. We analyzed the number of platelet transfusions between patients with ISPT and patients without ISPT as a surrogate for nonlethal severe hemorrhage. There was no difference comparing the group of patients without ISPT to the group of patients with ISPT with respect to the mean (2.9 versus 3 transfusions, respectively) or the median number of platelet transfusions (2 in each group). The increment in the blood platelet count 1 hour following transfusion was not significantly different between patients in the ISPT group (an increase of 34,000 platelet/μL) compared to controls (an increase of 29,000 platelet/μL; Table 2).

Table 2. *Comparison of Megakarocytes in Bone Marrow Biopsies and Response to Platelet Transfusions between Cases with ISPT and Patients Who Did Not Develop ISPT (Controls)*

	Cases ISPT	Controls No ISPT	*P*-value
Day 35 BM cellularity	**36% (26 patients)**	**37% (15 patients)**	**NS**
Day 35 megakaryocyte content in BM	**(25 patients)**	**(16 patients)**	**NS**
Increased	**16%**	**0%**	
Normal	**40%**	**56%**	
Decreased	**44%**	**44%**	
Posttransfusion platelet increment	**34,000/μL (7 patients)**	**29,000/μL (8 patients)**	**NS**

Bone marrow (BM) biopsies were performed at a median of day 34 (controls) or day 35 (ISPT) posttransplant in 6% and 42% of patients, respectively. The incremental change in the blood platelet count was measured 1 hour posttransfusion.
ISPT indicates idiopathic secondary posttransplant thrombocytopenia.

ISPT Was Significantly Associated with Posttransplant Survival

The median survival for the entire population was 6.2 years. Survival varied across diagnoses ($P = .03$), with median survivals for patients with acute myelogenous leukemia (AML) (N = 18) of 1.3 years, breast cancer (N = 34) of 6.2 years, Hodgkin's lymphoma (N = 59) of >6 years (median not reached), multiple myeloma (N = 164) of 5.5 years, non-Hodgkin's lymphoma (N = 80) of >6 years (median not reached), and other diagnoses (N = 4) (median not reached). Death was documented as having occurred in 100 (28%) of the 359 subjects in the study set at a median of 1.3 years posttransplant. Of the 78 patients with a documented cause of death, 69 (88%) died from their primary disease, 4 died from organ failure (5%), 3 died of infection (4%), and 1 each died of graft failure (1%) or hemorrhage (1%). We performed univariate Cox regression analyses to identify the clinical factors associated with posttransplant survival. Because of the very significant effect of diagnosis on survival (vide supra), these analyses were stratified by disease diagnosis. The status of the patients' primary malignancy at the time of transplant (complete remission [CR]; partial remission [PR]; or refractory), the number of prior chemotherapy regimens, the receipt of more than 2 platelet transfusions posttransplant, the failure to achieve a normal platelet count (>150,000/μL) in the initial 100 days posttransplant, and the occurrence of ISPT were significantly associated with worse posttransplant survival (Table 3). These covariates were then entered into a multivariable model of overall survival (OS), stratified by disease diagnosis. Four factors were independently associated with worse survival: the number of prior regimens, the disease status at the time of transplant, the failure to achieve a platelet count of >150,000/μL in the initial posttransplant engraftment period, and the occurrence of ISPT (Table 4). When the multivariate analysis was repeated without stratification on diagnosis, the failure to achieve a normal platelet count posttransplant, the occurrence of ISPT, and the disease status at the time of transplant remained independently associated with survival (Table 4).

Table 3. *Univariate Hazard Ratios for Death Posttransplant*

		95% CI		
	HR	**Lower**	**Upper**	***P*-value**
Age	**1.005**	**0.986**	**1.026**	**NS**
Sex	**1.068**	**0.676**	**1.687**	**NS**
Involved field radiation pretransplant	**2.212**	**0.813**	**6.022**	**NS**
Rituxan pretransplant	**0.044**	**0.000**	**29.636**	**NS**
Number of prior regimens	**1.532**	**1.054**	**2.227**	**.025**
Mobilization/harvest regimens (3 categoric variables)				**NS**
Number of aphereses	**1.095**	**0.838**	**1.430**	**NS**
Disease status at transplant	**2.1**	**1.4**	**3.0**	**<.001**
Conditioning regimens (categoric variables)				**NS**
$CD34^+$ cells per kg (3 strata)	**0.859**	**0.666**	**1.108**	**NS**
$CD34^+$ $CD38^-$ cells per kg (3 strata)	**0.919**	**0.719**	**1.173**	**NS**
Day of ANC(500) engraftment	**1.005**	**0.934**	**1.082**	**NS**
Day of PLT(50) engraftment	**1.012**	**0.998**	**1.026**	**NS**
Posttransplant platelet >150,000/μL	**0.433**	**0.281**	**0.666**	**<.001**
Cases of ISPT	**1.678**	**1.034**	**2.723**	**.036**
>2 platelet transfusions	**1.507**	**1.011**	**2.246**	**.044**

Cox regression analyses were performed after stratification by diagnosis. *P* values <.05 are shown.
ISPT indicates idiopathic secondary posttransplant thrombocytopenia; HR, hazard ratio; CI, confidence interval.

Table 4. *Multivariable Model with Hazard Ratios for Death Post-Transplant, Stratified by Disease Diagnosis*

	HR	95% CI Lower	95% CI Upper	*P*-value
Maximal initial posttransplant platelet count <150,000/μL*	**2.3**	**1.5**	**3.5**	**<.001**
ISPT*	**2.1**	**1.3**	**3.5**	**.003**
Number of prior regimens	**1.4**	**1.1**	**2.0**	**.022**
Disease status at transplant*	**2.0**	**1.4**	**2.9**	**<.001**

Factors that were significant in univariate analyses (Table 3, $P \leq .05$) were included in a forward conditional multivariate model, stratified by disease diagnosis.

CI indicates confidence interval; HR, hazard ratio; IPST, isiopathic secondary posttransplant thrombocytopenia.

*Three factors, a subnormal maximal platelet count posttransplant, ISPT, and disease status at transplant were also significantly associated with survival in a multivariate model in which the analysis was not stratified by disease diagnosis with HR (95%CI) of 2.3 (1.5-3.4), 1.9 (1.2-2.9), and 1.8 (1.3-2.4) respectively.

Development of a Thrombopoiesis Score for Posttransplant Survival

Figure 2 illustrates the effect on OS of the 3 covariates that were most significantly associated with survival in the multivariate model: the disease status at the time of transplant, the maximal platelet count measured during the initial engraftment period, and the presence of ISPT. Patients in PR or with refractory cancer at the time of transplant had worse survival than patients in CR ($P = .001$; Figure 2A). Patients who never achieved a platelet count of >150,000/μL at the time of initial engraftment had significantly worse OS than patients who achieved initial platelet counts ≥150,000 posttransplant ($P < .0001$), and pa-

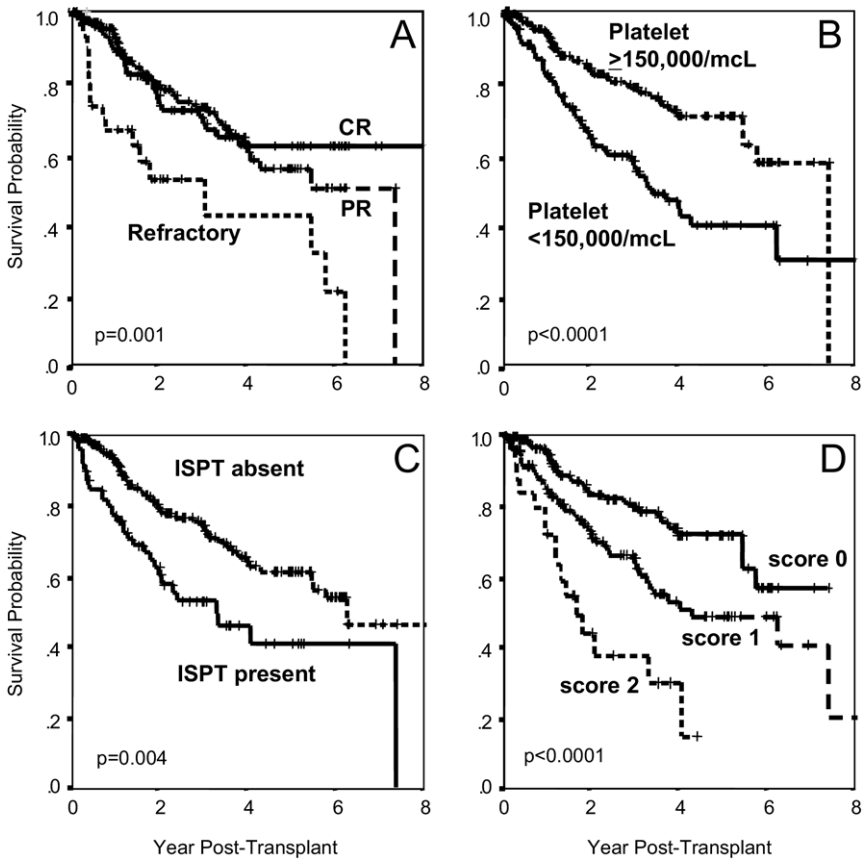

Figure 2. Kaplan-Meier estimates of overall survival of patients stratified by covariates associated with survival in the multivariate model. Survival curves are shown for patients stratified by A, remission status of their malignancy at the time of transplant, B, the achievement of a posttransplant platelet count of at least 150,000/μL and C, the presence of ISPT. *P* values based on log-rank comparisons are shown. D, The entire group of patients is stratified into 3 groups by the thrombopoiesis score, combining the effects seen in panels B and C by assigning 1 point each for the presence of a posttransplant platelet count of <150,000/μL and the occurrence of ISPT. All graphs represent outcome measurements on 359 patients except for panel A (358 patients; 1 patient with amyloidosis who was conditioned without prior therapy was excluded from the analysis).

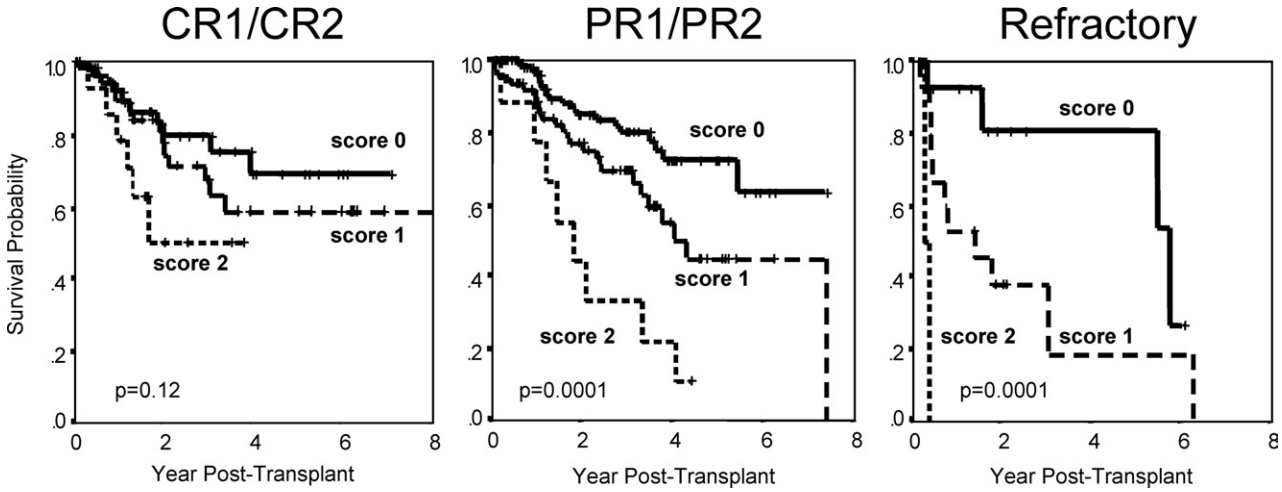

Figure 3. Kaplan-Meier estimates of overall survival of patients stratified by remission status at the time of transplant. Survival curves are shown for patients according to their remission status of their malignancy at the time of transplant. A, One hundred twenty-three patients transplanted in CR; B, 205 patients transplanted in PR; C, 30 patients transplanted with refractory disease. Each group is stratified by the thrombopoiesis score with *P*-values based on log-rank comparisons shown. Outcome data on 358 patients exclude the one patient with amyloidosis who was conditioned without prior therapy.

tients with ISPT had significantly worse survival than patients without ISPT ($P = .008$) (Figure 2B and C, respectively). Two covariates related to posttransplant thrombopoiesis in the multivariable analysis, namely, the failure to achieve a platelet count of >150,000 in the first 100 days posttransplant and the presence of ISPT, were used to generate a prognostic score (the "thrombopoiesis score"), in which 1 point was assigned for the presence of either factor, for a total score of 0, 1, or 2. One hundred eighty-nine patients had a prognostic score of 0 and have not reached a median survival; 145 patients had a score of 1 and have a median survival of 4.3 years; and 25 patients had a score of 2 and have a median survival of 1.7 years ($P < .001$ by log rank comparison; Figure 2D). The combination of low platelet counts at the time of initial engraftment and ISPT was associated with a very poor long-term survival, with an estimated 5-year survival of <15% for this subset (Figure 2D). Eighty-two percent of the deaths observed in the group with both low platelet counts and ISPT (in which a cause of death was recorded) resulted from the patients' primary disease. Application of this prognostic score to each of the different disease categories represented in the study set showed that the score was significantly associated with OS among patients in the 2 categories with the largest numbers of patients: multiple myeloma (N = 164, $P < .0001$), and non-Hodgkin's lymphoma (N = 80, $P = .01$).

Significance of the Thrombopoiesis Score on OS after Stratification on Disease Status at the Time of Transplant

To address the question of the relationship of posttransplant thrombopoiesis with disease status, we repeated the multivariate analysis stratifying on remission status at the time of transplant. The occurrence of ISPT ($P = .002$), the failure to achieve a platelet count of >150,000 ($P < .001$), the number of prior chemotherapy regimens ($P = .019$), and the diagnosis ($P = .01$) were significant covariates associated with death. We analyzed the significance of the "thrombopoiesis score" on the entire population stratified by their pretransplant remission status and present these data graphically in Figure 3. As expected, the 123 patients who were in CR1 or CR2 at the time of transplant had the best OS, and although the thrombopoiesis score separated the survival curves of this group, the differences were not significant by log-rank test ($P = .12$). Among the large group of 205 patients transplanted with chemosensitive disease in PR, the thrombopoiesis score produced a very significant stratification in survival ($P < .0001$). Among the smaller number of 30 patients transplanted with refractory disease (mostly patients with refractory Hodgkin's lymphoma or multiple myeloma) the thrombopoiesis score again separated these populations into groups with statistically different survival experiences posttransplant ($P = .0001$).

Association of the Covariates in the Multivariate Model with Engraftment Kinetics and Numbers of Transplanted Progenitor Cells

We compared the mean values for days to granulocyte and platelet engraftment and the numbers of transplanted $CD34^+$ and $CD34^+$ $CD38^-$ cells across each of the covariates in the multivariate model to test (1) whether these clinical factors (including ISPT and low initial platelet counts) were significantly associated with the kinetics of engraftment, and (2) whether

Table 5. *Risk Factors for Posttransplant Death in the Multivariable Model Were Associated with Delayed Platelet Engraftment and Lower Numbers of Total CD34$^+$ Cells and CD34$^+$ CD38$^-$ Cells in the Hematopoietic Graft*

	Days to ANC >500/μL			Days to Platelet >50,000/μL			CD34$^+$ (x10E6)/kg			CD34$^+$ CD38$^-$ (x10E6)/kg		
	Mean	SEM	*P*-value	Mean	SEM	*P*-value	Mean	SEM	*P*-value	Mean	SEM	*P*-value
ISPT												
Present (62)	12.7	0.2	NS	18.4	0.7	NS	8.8	0.9	NS	0.076	0.010	.0006*
Absent (297)	13.4	0.2		21.1	0.7		10.3	0.8		0.134	0.014	
Maximal platelets												
<150 K/μL (133)	13.3	0.3	NS	26.5	1.3	<.0001	7.3	0.7	<.0001*	0.142	0.024	NS
≥150 K/μL (226)	13.2	0.2		17.2	0.5		11.7	0.9		0.113	0.011	
Status at transplant												
CR (123)	13.0	0.2	NS	20.9	1.0	NS	10.1	1.3	NS	0.130	0.021	NS
PR (205)	13.4	0.2		20.6	0.8		9.6	0.6		0.116	0.012	
REF (30)	13.2	0.8		20.5	1.7		12.9	3.6		0.150	0.064	
Prior regimens												
≤1 (186)	13.4	0.2	NS	20.6	0.8	NS	9.9	0.7	NS	0.102	0.01	.05

Mean values with standard error of the mean (SEM) for the number of days to achieve myelogenous engraftment, platelet engraftment, and the content (per kg) of CD34$^+$ and CD34$^+$CD38$^-$ cells in the graft were calculated. Patients were stratified into groups by the dichotomous covariates identified in the in multivariable model (ISPT and failure to achieve a normal platelet count post-transplant) or according to a cut point greater than the median number of prior regimens (median of 1). Statistical comparison between means was performed using the *t*-test. Nonequal variances were used.

ANC indicates absolute neutrophil count; CR, complete remission; PR, partial remission.

they were associated with transplantation of fewer hematopoietic progenitor cells. Platelet and granulocyte engraftment kinetics did not vary significantly between patients who received 1, 2, 3, or 4 prior regimens (data not shown), but did vary according to the content of CD34$^+$ cells in the graft. More rapid engraftment occurred among the third of patients receiving the highest number of CD34$^+$ cells/kg achieved platelet count (50,000/μL) at a median of 17 days compared to 18 days among the third of patients receiving the lowest number of CD34$^+$ cells/kg ($P < .0001$). Patients who failed to achieve a posttransplant platelet count of >150,000/μL received significantly fewer numbers of transplanted CD34$^+$ cells/kg ($P < .0001$, Table 5), whereas patients with ISPT received equivalent numbers of total CD34$^+$ cells but fewer CD34$^+$CD38$^-$ cells/kg (0.076 × 10E6/kg) than patients who did not develop ISPT (0.13 × 10E6/kg, $P = .001$, Table 5).

DISCUSSION

This is the largest single-institution retrospective study on the kinetics of platelet engraftment following autologous HPCT that we are aware of, and provides some interesting observations. First, the phenomenon of ISPT appears relatively common, occurring in 17% of patients. Second, although most patients recovered from secondary thrombocytopenia, the subset of patients who never achieved a normal platelet count posttransplant and then had a secondary decline in platelet counts following initial engraftment had a very poor long-term outcome, with most deaths from relapse of their primary disease (Figure 2). A lower platelet count at the time of initial engraftment was associated with transplantation of fewer numbers of total CD34$^+$ cells, whereas the occurrence of ISPT was associated with transplantation of fewer CD34$^+$ CD38$^-$ cells. These data suggest that both the quantity and quality of autologous hematopoietic progenitor cells in the graft are important prognostic variables for long-term survival, in addition to being markers for initial hematopoietic engraftment.

One question raised by this analysis is why posttransplant thrombopoiesis is associated with long-term survival in this group of patients. Because we limited the analysis to patients who had engrafted with platelet counts of >50,000/μL, the association of measures of posttransplant thrombopoiesis with survival is not because of death from graft failure or very early regimen-related toxicities. We think that the thrombopoiesis score is a surrogate for the activity of the underlying malignancy in patients following high-dose chemotherapy and autologous HPCT. In this retrospective review of 359 patients, the schedule for posttransplant restaging varied according to the diagnosis, and occurred at various times between day 30 and 6 months posttransplant. Although controlling for the remission status achieved posttransplant in our analysis was not practical, the disease status at the time of transplant provides an indication as to the chemosensitivity of the underlying malignancy. The occurrence of ISPT and the maximal platelet count achieved in the initial period of engraftment posttransplant were combined into a thrombopoiesis score that significantly associated with posttransplant OS of all patients (Figure 2D), and most significantly associated with survival among patients transplanted in PR or with refractory cancer (Figure 3). Thus, the asso-

ciation of the thrombopoiesis score with survival among patients beginning conditioning with evidence of residual disease (PR or refractory groups), and the fact that 88% of posttransplant deaths were from disease progression suggests that impaired postengraftment thrombopoiesis reflects failure of the high-dose chemotherapy to eradicate residual disease in the patient. Because ISPT and lower platelet counts during initial engraftment were both independent covariates with OS in a multivariate model (Table 4), the thrombopoiesis score is not simply a surrogate for the extent of prior therapy. One speculative hypothesis is that tumor stem cells and normal hematopoietic stem cells compete for the same niches in hematopoietic microenvironments. As markers for tumor stem cells become more refined, this may be a testable hypothesis if accessible sites, such as the bone marrow, could be sampled at various time points before and after stem cell transplantation.

There was a significant association between the failure to achieve an initial platelet count of >150,000/μL with a lower dose of total $CD34^+$ cells in the graft, as well as an association of ISPT with grafts containing fewer $CD34^+$ $CD38^-$ cells (Table 5). The minimum quantity of $CD34^+$ cells required for consistent engraftment is unknown, but is probably between 1-2 × 10E6 cells/kg [3,21] and predicts the kinetics for engraftment of granulocyte and platelets following autologous transplantation [5,22,23]. As expected, we observed slower rates for initial platelet engraftment among those patients who received fewer $CD34^+$ cells in their graft. Because the study set was limited to patients who achieved initial platelet engraftment, the association of a lower $CD34^+$ cell dose with the kinetics of platelet engraftment was not from the inclusion of patients who failed to engraft. Of note, patients with ISPT had a shorter time to reach a transfusion-independent platelet count of 50,000/μL (Table 5), indicating that ISPT was not primarily associated with the kinetics of the initial engraftment.

Subsets of $CD34^+$ cells have been reported to predict the kinetics of early platelet engraftment [24]. Our study also confirmed a modest effect of the dose of $CD34^+$ $CD38^-$ cells on early platelet engraftment, although the effect of the total $CD34^+$ cell dose was more significant in a multivariate model (data not shown). The phenomena of ISPT may be related to patients receiving a smaller number of long-term repopulating stem cells that are contained in the $CD34^+$ $CD38^-$ subpopulation. In murine and sheep models, only recipients transplanted with $CD34^+$ $CD38^-$ cells (but not those with $CD34^+$ $CD38^+$ cells) have been shown to exhibit long-term engraftment during serial transplantations [20], and the $CD34^+$ $CD38^-$ subset may represent the long-term repopulating cells responsible for sustained engraftment [25]. Although the optimum number of $CD34^+$ $CD38^-$ cells in the graft to achieve rapid and durable hematopoietic reconstitution has been defined as $>5 \times 10^4$ cells/kg body weight [13,26,27], our analyses indicate that higher numbers are insufficient, in some cases, for stable engraftment. A possible explanation for the association of fewer numbers of $CD34^+$ $CD38^-$ cells with the occurrence of ISPT and the association of total $CD34^+$ cells with the maximal platelet count measured during the initial engraftment period is from the presence of long-term repopulating stem cells in the former population, and the presence of committed progenitors in the later population. Alternatively, the $CD34^+$ $CD38^-$ subset of the hematopoietic graft could contain stromal progenitors [28] that may be important in facilitating thrombopoiesis in the marrow microenvironment [29].

We did not find a direct association between the quantity of $CD34^+$ or $CD34^+CD38^-$ cells transplanted and survival in this study (Table 2). These findings are in contrast to studies of allogeneic transplantation, in which greater numbers of $CD34^+$ cells in a marrow (but not blood HPC) graft are associated with improved survival [30,31]. Thus, measurements of the number of $CD34^+$ cells or $CD34^+$ $CD38^-$ cells in the autologous HPC graft are an imperfect assessment of stem cell quality and do not fully predict hematopoiesis.

Finally, we did not demonstrate an autoimmune etiology for ISPT observed in patients undergoing autologous HPCT. We found equivalent numbers of bone marrow megakaryocytes in day +35 marrow samples from patients with ISPT compared to bone marrow biopsies from patients without ISPT (Table 3); equivalent posttransfusion increments in blood platelet counts comparing the 2 groups (Table 3); and a lack of therapeutic response of blood platelet count to trials of immunosuppressive therapies that included an average of more than 40 mg prednisone/day for a month in a subset of ISPT patients (Figure 1C). We suggest avoidance of immunosuppressive drug therapy in patients who do not have evidence for IPT.

ACKNOWLEDGMENTS

The authors acknowledge Drs. Rein Saral, Amelia Langston, and Sagar Lonial for helpful discussions, and Dr. Michael Graiser, Zahir Ali, Pareen Kamat, Anisha Philip, and Kevin Bumpers for assistance in data acquisition.

REFERENCES

1. Weaver CH, Hazelton B, Birch R, et al. An analysis of engraftment kinetics as a function of the CD34 content of peripheral blood progenitor cell collections in 692 patients

after the administration of myeloablative chemotherapy. *Blood*. 1995;86:3961-3969.
2. Siena S, Schiavo R, Pedrazzoli P, et al. Therapeutic relevance of CD34 cell dose in blood cell transplantation for cancer therapy. *J Clin Oncol*. 2000;18:1360-1377.
3. Shpall EJ, Champlin R, Glaspy JA. Effect of CD34+ peripheral blood progenitor cell dose on hematopoietic recovery. *Biol Blood Marrow Transplant*. 1998;4:84-92.
4. Bensinger W, Appelbaum F, Rowley S, et al. Factors that influence collection and engraftment of autologous peripheral-blood stem cells. *J Clin Oncol*. 1995;13:2547-2555.
5. Schwartzberg L, Birch R, Blanco R, et al. Rapid and sustained hematopoietic reconstitution by peripheral blood stem cell infusion alone following high-dose chemotherapy. *Bone Marrow Transplant*. 1993;11:369-374.
6. Kiss JE, Rybka WB, Winkelstein A, et al. Relationship of CD34+ cell dose to early and late hematopoiesis following autologous peripheral blood stem cell transplantation. *Bone Marrow Transplant*. 1997;19:303-310.
7. Klaus J, Herrmann D, Breitkreutz I, et al. Effect of CD34 cell dose on hematopoietic reconstitution and outcome in 508 patients with multiple myeloma undergoing autologous peripheral blood stem cell transplantation. *Eur J Haematol*. 2007;78: 21-28.
8. Porrata LF, Litzow MR, Inwards DJ, et al. Infused peripheral blood autograft absolute lymphocyte count correlates with day 15 absolute lymphocyte count and clinical outcome after autologous peripheral hematopoietic stem cell transplantation in non-Hodgkin's lymphoma. *Bone Marrow Transplant*. 2004;33: 291-298.
9. Porrata LF, Gertz MA, Geyer SM, et al. The dose of infused lymphocytes in the autograft directly correlates with clinical outcome after autologous peripheral blood hematopoietic stem cell transplantation in multiple myeloma. *Leukemia*. 2004;18: 1085-1092.
10. Porrata LF, Litzow MR, Tefferi A, et al. Early lymphocyte recovery is a predictive factor for prolonged survival after autologous hematopoietic stem cell transplantation for acute myelogenous leukemia. *Leukemia*. 2002;16:1311-1318.
11. Porrata LF, Inwards DJ, Micallef IN, et al. Early lymphocyte recovery post-autologous haematopoietic stem cell transplantation is associated with better survival in Hodgkin's disease. *Br J Haematol*. 2002;117:629-633.
12. Porrata LF, Gertz MA, Inwards DJ, et al. Early lymphocyte recovery predicts superior survival after autologous hematopoietic stem cell transplantation in multiple myeloma or non-Hodgkin lymphoma. *Blood*. 2001;98:579-585.
13. Waller EK, Lynn M, Worford L, et al. The number of CD34+ CD38− cells in the graft predicts the kinetics of hematopoietic reconstitution following autologous or allogeneic *BMT Blood*. 1997;90:394.
14. Schuster MW, Beveridge R, Frei-Lahr D, et al. The effects of pegylated recombinant human megakaryocyte growth and development factor (PEG-rHuMGDF) on platelet recovery in breast cancer patients undergoing autologous bone marrow transplantation. *Exp Hematol*. 2002;30:1044-1050.
15. Hassan HT, Zander AR. Thrombocytopenia after high-dose chemotherapy and autologous stem cell transplantation: an unresolved problem and possible approaches to resolve it. *J Hematother*. 1996;5:407-414.
16. Hequet O, Salles G, Ketterer N, et al. Autoimmune thrombocytopenic purpura after autologous stem cell transplantation. *Bone Marrow Transplant*. 2003;32:89-95.
17. Jillella AP, Kallab AM, Kutlar A. Autoimmune thrombocytopenia following autologous hematopoietic cell transplantation: review of literature and treatment options. *Bone Marrow Transplant*. 2000;26:925-927.
18. Henon P, Sovalat H, Becker M, et al. Primordial role of CD34+ 38− cells in early and late trilineage haemopoietic engraftment after autologous blood cell transplantation. *Br J Haematol*. 1998;103:568-581.
19. Waller EK, Olweus J, Lund-Johansen F, et al. The "common stem cell" hypothesis reevaluated: human fetal bone marrow contains separate populations of hematopoietic and stromal progenitors. *Blood*. 1995;85:2422-2435.
20. Zanjani ED, Almeida-Porada G, Ascensao JL, et al. Transplantation of hematopoietic stem cells in utero. *Stem Cells.* 15(Suppl 1):79-92 [discussion 93].
21. Demirer T, Buckner CD, Bensinger WI. Optimization of peripheral blood stem cell mobilization. *Stem Cells*. 1996;14:106-116.
22. Rybka WB, Kiss JE, Lister J, et al. Graft CD34+ cell content predicts engraftment following allogeneic and autologous hematopoietic stem cell transplantation. *Blood*. 1994;84:338a.
23. Fernandez HF, Noto TA, Morrell L, et al. Early engraftment of mobilized autologous peripheral blood progenitor cells collected by large volume leukapheresis. *Blood*. 1994;84:365a.
24. Kamel AM, El-Sharkawy N, Mahmoud HK, et al. Impact of CD34 subsets on engraftment kinetics in allogeneic peripheral blood stem cell transplantation. *Bone Marrow Transplant*. 2005; 35:129-136.
25. Jones RJ, Wagner JE, Celano P, et al. Separation of pluripotent haematopoietic stem cells from spleen colony-forming cells. *Nature*. 1990;347:188-189.
26. Henon PH, Sovalat H, Bourderont D. Importance of CD34+ cell subsets in autologous PBSC transplantation: the mulhouse experience using CD34+CD38− cells as predictive tool for hematopoietic engraftment. *J Biol Regul Homeost Agents*. 2001; 15:62-67.
27. Drake M, Ranaghan EA, Alexander HD, et al. CD34+CD38− HLA-DR- correlate more closely than CD34+CD38− with engraftment post-PBSC transplantation. *Br J Haematol*. 1999; 104:643-644.
28. Waller EK, Huang S, Terstappen L. Changes in the growth properties of CD34+, CD38− bone marrow progenitors during human fetal development. *Blood*. 1995;86:710-718.
29. Guerriero A, Worford L, Holland HK, et al. Thrombopoietin is synthesized by bone marrow stromal cells. *Blood*. 1997;90: 3444-3455.
30. Waller EK, Rosenthal H, Jones TW, et al. Larger numbers of CD4(bright) dendritic cells in donor bone marrow are associated with increased relapse after allogeneic bone marrow transplantation. *Blood*. 2001;97:2948-2956.
31. Przepiorka D, Smith TL, Folloder J, et al. Risk factors for acute graft-versus-host disease after allogeneic blood stem cell transplantation. *Blood*. 1999;94:1465-1470.